SYMPOSIUM: MASTITIS IN DAIRY HEIFERS

Mastitis in Dairy Heifers: Initial Studies on Prevalence and Control

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ABSTRACT

Initial studies to determine the prevalence of mastitis in heifers of breeding age and in pregnant dairy heifers demonstrated that IMI were present in 97% of heifers and 75% of quarters. The most common isolates were Staphylococcus aureus, Staphylococcus hyicus, and Staphylococcus chromogenes; SCC ranged from 12.4 to 17.3 x 10^6/ml. Approximately 29% of heifers and 15% of quarters exhibited clinical mastitis at breeding age, as evidenced by clots or flakes in mammary secretions. Histologic examination of mammary tissues demonstrated significant reductions in alveolar epithelial and luminal areas and increases in connective tissue stroma and leukocytosis, illustrating limited development and marked inflammation of infected tissues. A one-time infusion of antibiotic for nonlactating cows into infected quarters 245 d prepartum reduced incidence of IMI by 59% at calving compared with the pretreatment level; the cure rate for Staph. aureus IMI was >90%. Prophylactic treatment of uninfected quarters 245 d prepartum reduced new Streptococcus sp. IMI by 93%. The mean SCC was 50% lower at calving for treated heifers, and milk yield over the first 2 mo of lactation was 10% greater than that of untreated controls. Heifers from herds using fly control had a lower prevalence of IMI than herds without fly control. Prevalences of IMI and SCC in dairy heifers were higher than previously realized, but mastitis at calving was controlled by use of therapeutic products for nonlactating cows during pregnancy.

(Key words: heifer, mastitis, dry cow therapy)

Abbreviation key: CNS = coagulase-negative staphylococci.

INTRODUCTION

Current methods of mastitis control advocate the adoption of management practices that were developed for mature lactating and dry cows. These include pre- and postmilking teat dipping, milking of clean and dry teats, proper use of functionally adequate milking machines, prompt treatment of clinical cases, and culling of chronically infected animals.

Management practices and disease control for heifers include proper housing, adequate nutrition, AI, and vaccination against calfhood diseases, with little or no concern about mastitis. However, heifers so managed do become infected, and, in some herds, the overall mastitis level is 97% of the animals; Staphylococcus aureus causes 37% of these infections (49). Heifer mastitis is not to be confused with “summer mastitis”, which is caused by Actinomyces pyogenes, Peptococcus indolicus, or Streptococcus dysgalactiae and is spread by the fly, Hydrotaea irritans (56).

Intramammary infections during the first lactation are prevalent in many herds, ranging from 10.4 to 64% (7, 29, 30, 35, 41). Presence of IMI at this time may be attributed to feeding calves unpasteurized mastitic milk and allowing calves to suckle one another (27). Heifers infected before parturition occasionally freshen with one or more nonfunctional quarters, and first lactation yield may be diminished (40).
one study (30), almost 60% of mammary quarters infected with staphylococci during the first lactation continued to shed these organisms through the second lactation.

The mammary glands of unbred and primigravid heifers have traditionally been regarded as uninfected and are not examined until the first milking or during the first episode of clinical mastitis following calving. Presence of mammary inflammation in young dairy heifers could be deleterious to future milk yield. The greatest mammary tissue development occurs during the first gestation (2, 52), and presence of IMI could adversely affect secretory cell differentiation. Boddie et al. (3) found that mammary parenchymal tissue from unbred heifers showed inflammatory responses in infected quarters. King (23) compared milk yield and composition of previously infected quarters with contralateral, uninfected quarters in first lactation cows and found that milk yield was decreased 18%; milk fat and SNF from infected quarters were also reduced.

Mammary secretory tissues and teat canal keratin become colonized with mastitis-causing bacteria well before heifers freshen; these bacteria persist for up to 1 yr (3), and secretory potential could be compromised at critical stages in mammary development. Thus, mammary glands must be protected from the harmful effects of mastitis-causing bacteria to ensure maximum milk yield during the first lactation.

**RESEARCH HERD STUDIES ON HEIFER MASTITIS**

**Distribution of Mastitis-Causing Microorganisms and Persistence of IMI**

The prevalence of mastitis in 10 unbred Jersey heifers (10 to 12 mo of age) at the Hill Farm Research Station was initially evaluated in 1985 and was monitored over a 1-yr period, which covered breeding age and gestation (3). Sampling of teat skin, teat canal, and secretion was performed bimonthly and continued through freshening. Samples from teat skin were collected by swabbing a 1-cm² area of the midteat with a rayon-tipped swab (Culturette®; Becton Dickinson, Cockeysville, MD) and were stored in .5 ml of modified Stuart’s transport medium (Becton Dickinson) until plated. For samples from the teat canal, teat ends were scrubbed with cotton balls moistened with 70% isopropyl alcohol. A calcium alginate nasal swab on an ultrafine aluminum shaft (Calgiswab, Type 4: Fisher Scientific, Pittsburgh, PA) was inserted 2 to 3 mm into the teat canal, removed, and placed in .5 ml of sterile physiological saline. After teat ends were resanitized, samples of secretion from quarters containing fluid were collected in sterile culture tubes. All quarters from which secretions were collected prepartum were dipped with a barrier teat dip containing 1% lauricidin in an acrylic latex base (Teat Shield™ with Germicide: 3M Company, St. Paul, MN) following sample collection. After heifers freshened, samples of secretion were collected prior to milking and were taken every 2 wk during lactation. Somatic cell counts were performed using a Fossomatic cell counter (A/SN Foss, Hillerød, Denmark).

Microbiological procedures were followed as described (15). Samples of teat skin were plated on eosin methylene blue agar for isolation of coliforms, thallium crystal violet toxin agar (Becton Dickinson) for streptococci, and mannitol salt agar (Difco Laboratories, Detroit, MI) for staphylococci. Samples of teat canal and secretion were plated on tryptose blood agar (Difco Laboratories) containing 5% calf blood and .1% esculin. Organisms were identified presumptively by colony morphology and hemolysin production. Staphylococcal isolates were tested for coagulase production and for Gram and catalase reactions (15). Species identification of staphylococci was performed using the modified API Staph-Ident System (Analytab Products, Plainview, NY) (54). Streptococcal isolates were tested for esculin hydrolysis, CAMP reaction, bile-esculin, hydrolysis, penicillin susceptibility, and utilization of inulin, raffinose, trehalose, arabinose, and sorbitol. The most frequently isolated organisms from teat skin were coliforms; excessive numbers precluded identification to the species level. Coliforms were never isolated from teat canals or secretions. These organisms were considered to be contaminants, not normal flora. Eberhart (11) also found that prevalence of coliform colonization on teat ends was low compared with prevalence of staphylococci and streptococci, and, when large numbers of coliforms were present, they resulted
from environmental contamination. Therefore, after several samplings, culture procedures for isolation of coliforms were discontinued.

For bacteriologic analysis, a total of 388 samples from teat skin, 388 from teat canal, and 216 from secretion were examined. Bacteria (staphylococci and streptococci) were isolated from 54.1, 70.1, and 86.1% of samples from teat skin, teat canal, and secretion, respectively. *Staphylococcus xylosus* and *Staph. chromogenes* were the predominant flora isolated from teat skin, followed by *Staph. warneri, Staph. sciuri, Staph. aureus, Staph. hyicus,* and *Staphylococcus simulans.* The most prevalent coagulase-negative staphylococci (CNS) found in teat canals were *Staph. chromogenes,* followed by *Staph. hyicus, Staph. aureus, Staph. xylosus, Staph. warneri,* and *Staph. sciuri.* In mammary secretions, *Staph. chromogenes* was the predominant organism, followed by *Staph. hyicus,* *Staph. aureus,* *Streptococcus uberis,* and *Staph. xylosus.* These bacteria were present at the first sampling and were isolated in secretions from individual quarters with each successive sampling of the trial.

The most prevalent CNS isolated from teat skin, *Staph. xylosus,* has previously been found to be a major species occurring on normal skin of cattle and other ungulates (26). *Staphylococcus sciuri* has also been found on cattle and other mammalian skin (25). Devriese (8) also found these CNS organisms in milk and teat canals and on teat apices and teat skin of cows. In the present study, there appeared to be a correlation between colonization of the teat canal and IMI. For example, the major species colonizing the teat canal (*Staph. chromogenes, Staph. hyicus, Staph. aureus,* and *Staph. xylosus*) were also the predominant organisms causing IMI. Similarly, Forbes and Hebert (13) observed that, for lactating cows, the majority of *Staph. aureus* and *Staph. epidermidis* (CNS) IMI were preceded by teat canal colonization by these organisms, and those researchers concluded that chronic IMI may be maintained by the presence of teat canal colonization.

*Streptococcus uberis* has been isolated in large numbers from teat apices of nonlactating cows (44), and, in some herds, a high percentage of first lactation heifers freshened with streptococcal IMI at parturition (29, 36). In contrast, *Strep. uberis* was isolated from only 3 samples of secretion in the present research herd study (1.4%) and never on teat apices.

Because a substantial percentage of first lactation cows are infected with primary pathogens at calving, such as *Staph. aureus,* environmental streptococci, and *Escherichia coli* (29), examination of the microflora of heifers at an early age is warranted. This initial study indicated that teat skin and canals and mammary secretions of heifers are colonized with CNS, as well as *Staph. aureus,* at an early age and that the infections may persist for up to 1 yr. Species identification demonstrated that nearly all isolates from the same quarter throughout the study were the same biovariant, which supports the contention that isolates from each quarter over time were from persistent infections and not from new IMI occurring between sampling periods. Although the percentage of *Staph. aureus* isolates was low in relation to CNS, presence of this major pathogen demonstrated that it colonized teat canals at a much earlier age than documented previously (43).

### Leukocyte Response to IMI

Milk SCC are considered to be an important parameter for assessing mammary health status in lactating cows, and milk yield decreases as SCC and incidence of mastitis increase (22, 24). Thus, SCC in heifer mammary gland secretions were analyzed to measure the degree of inflammation and potential reductions of future milk yield. The SCC of secretion samples collected during the study were determined when the volume and consistency of the sample permitted. The mean SCC of quarters infected with *Staph. chromogenes, Staph. hyicus,* and *Staph. aureus* were 7.8, 8.5, and 9.2 $\times 10^6$, respectively. The mean SCC of uninfected quarters was 3.5 $\times 10^6$. The mean SCC of heifer secretions collected on the day of freshening were 3.2 and 1.6 $\times 10^6$ for quarters infected by staphylococcus and uninfected quarters, respectively. The mean SCC during the first 3 mo of lactation in quarters infected with *Staph. chromogenes, Staph. hyicus,* and *Staph. aureus* were 168, 193, and 578 $\times 10^3$, respectively; SCC of uninfected quarters was 39 $\times 10^3$. The SCC for quarters infected with CNS were slightly lower than those reported
by Harmon and Langlois (17) but comparable with those of Watts and Owens (53). Thus, the present study found SCC approaching 200 × 10^3 for quarters infected with CNS during the first 3 mo of lactation, and, based on previous studies (22, 24), SCC in this range are associated with milk loss. Surprisingly, 13% of quarter secretions sampled prepartum contained Staph. aureus. After freshening, the SCC of quarters infected with Staph. aureus averaged 578 × 10^3/ml, a cell count that was associated with a loss of >2 kg of milk/d (24).

### Influence of IMI on Mammary Gland Histology

The mammary glands of two heifers were studied initially to determine histological responses to teat canal colonization with Staph. chromogenes and Staph. hyicus (3). The heifers were slaughtered at 18 mo of age, and mammary tissues were processed for microscopy as described (34). Histological examination of mammary tissues from both heifers demonstrated a leukocyte reaction to the colonization of the teat canal. Cross-sections through the midteat canal demonstrated cocci colonizing keratinized cells of the canal lumen, and sections of distal teat cisternal tissues demonstrated heavy leukocyte infiltration with lymphocytes and plasma cells at Fürstenberg's rosette compared with uninfected tissues. Presence of lymphoid cells in this region may be a result of contact with bacterial antigens and subsequent proliferation and differentiation. Du Preez (9) suggested that bacteria colonizing the teat canal produce toxins that diffuse to the interior of the udder, inflicting damage to milk-producing cells.

Seven unbred heifers, 14 to 26 mo of age, were studied subsequently to evaluate the effects of IMI on leukocyte infiltration and characteristics of secretory tissue in developing mammary glands (48). Gross observations of mammary gland tissues from heifers demonstrated the presence of an adipose tissue pad on the dorsal surface of the gland, and, ventral to this pad, lobes of developing secretory tissue were observed throughout adipose tissue. Histologic observations of tissue samples from lobes of mammary parenchyma of uninfected quarters showed that alveoli were small; the epithelial lining was composed of a single layer of cuboidal cells surrounding a small luminal space with little or no stained secretory product. Interalveolar connective tissue area composed approximately half of the observed lobes, and a few infiltrating leukocytes, mainly lymphocytes, were observed.

Infected tissues, particularly those with Staph. aureus IMI, exhibited large amounts of interalveolar connective tissues and reductions in epithelial and luminal areas. Such areas also exhibited leukocytic infiltration, particularly lymphocytes and neutrophils, into stromal and luminal areas. Hyperplasia of ducts and cisterns as a result of infection was also observed, and macro- and microscopic abscesses were found in the parenchyma of one quarter infected with Staph. aureus. Some of the abscesses were tubercule-like with a circular, stratified fibrosis containing numerous lymphocytes, neutrophils, plasma cells, and multinucleated giant cells.

Results of morphometric analysis on parenchymal tissue components (48) showed that percentages of each component in uninfected quarters were very similar to percentages from quarters infected with CNS, although quarters infected with CNS exhibited significantly more stromal area (Table 1). Percentages of alveolar

### Table 1. Morphometric analysis of mammary parenchyma from uninfected and infected quarters of seven unbred Jersey heifers 14 to 26 mo of age.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Uninfected quarters</th>
<th>Coagulase-negative staphylococci</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelium, %</td>
<td>30.0^a</td>
<td>28.3^a</td>
<td>26.4^b</td>
</tr>
<tr>
<td>Lumen, %</td>
<td>11.3^a</td>
<td>9.5^a</td>
<td>6.5^b</td>
</tr>
<tr>
<td>Stroma, %</td>
<td>58.7^c</td>
<td>62.2^c</td>
<td>67.1^c</td>
</tr>
</tbody>
</table>

^a,b,c^Means with different superscripts within each row differ (P < .05).

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epithelium and lumen in quarters infected with Staph. aureus were significantly lower \((P < .05)\) than those in uninfected quarters and in quarters infected with CNS. Quarters infected with Staph. aureus also showed a greater percentage \((P < .05)\) of interalveolar stroma than did uninfected quarters and quarters infected with CNS.

Previous histologic studies of lactating and dry cows have demonstrated the deleterious effects of Staph. aureus IMI on mammary parenchymal tissues. Heald (18) and Nickerson and Heald (33) reported that quarters infected with Staph. aureus demonstrated greater interalveolar stromal areas and fewer areas of secretory epithelium and alveolar lumen than did uninfected quarters, suggesting a reduction in the ability of affected tissues to synthesize and secrete milk. Another histologic study involving dry cows (46) reported that Staph. aureus IMI did not affect the percentage of epithelial tissues but accelerated the involution process, as evidenced by increased nonsecretory epithelium and interalveolar stroma and reduced alveolar luminal areas. These observations about mature lactating and dry cows suggest that similar histopathological changes could occur in heifers. The greatest development of secretory tissue in these young heifers occurs during the first pregnancy, and developing secretory tissues may be affected by infection, leading to deposition of connective tissue stroma instead of milk secretory tissue and subsequent deleterious effect on future milk yield.

Leukocyte infiltration into cisternal and parenchymal mammary tissues are presented in Table 2. Quarters infected with Staph. aureus exhibited the greatest tissue leukocytosis, followed by quarters infected with CNS and uninfected quarters. Leukocyte infiltration in gland cistern and secretory tissue for infected quarters was significantly higher \((P < .05)\) than that for uninfected quarters. Leukocytosis into teat cistern tissue was similar for uninfected quarters and those infected with CNS, but significantly lower \((P < .05)\) than quarters with Staph. aureus IMI. None of the uninfected quarters or quarters infected with CNS demonstrated marked leukocyte infiltration. However, marked leukocyte infiltration, particularly lymphocytes, into cisternal and parenchymal areas was commonly observed in quarters that were infected with Staph. aureus. The majority of leukocytes observed within lumens were neutrophils. Several studies (1, 5, 16, 21) have reported that neutrophil migration into parenchymal tissues, as well as the phagocytosis process, may lead to lysis of mammary secretory tissue of lactating cows, and this damage may be related to decreases in milk production. Furthermore, Huston and Heald (21) suggested that the affected tissue is not repaired until the next dry period. Whether this damage might affect future milk yield in heifers needs to be ascertained.

Results of these initial studies demonstrated that teat canal colonization and IMI in unbred heifers occurred as early as 10 mo of age and persisted into the first lactation. The SCC information collected from quarters prepartum and during early lactation demonstrated that leukocyte concentrations from glands infected with CNS and Staph. aureus were similar to

<table>
<thead>
<tr>
<th>TABLE 2. Leukocyte infiltration in mammary tissue from uninfected and infected quarters of 7 unbred Jersey heifers 14 to 26 mo of age.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tissue</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Teat cistern lining</td>
</tr>
<tr>
<td>Gland cistern lining</td>
</tr>
<tr>
<td>Gland cistern parenchyma</td>
</tr>
<tr>
<td>Deep parenchyma</td>
</tr>
</tbody>
</table>

*Means with different superscripts within each row differ \((P < .05)\).

Expressed as mean leukocyte infiltration score, where 1 = none to a few leukocytes observed, 2 = moderate leukocyte infiltration, and 3 = marked leukocyte infiltration.

those found previously to be associated with reduced milk yield. In addition, histological study of udder tissue revealed inflammatory responses in infected quarters long before freshening, including leukocytosis and marked reductions in luminal area and increases in connective tissue stroma, which may be detrimental to parenchymal development.

HEIFER MASTITIS IN COMMERCIAL HERDS

Prevalence of Teat Canal Colonizations, Mastitis, SCC, and Differential Leukocyte Counts

Studies were designed next to measure the prevalence of mastitis in heifers of breeding age and pregnant heifers in commercial herds and to determine whether intramammary treatment during pregnancy could be effective in reducing the level of mastitis at calving (49, 50, 51). Because bacteria from the environment, from flies, or from other heifers appear to colonize teat canal keratin as a prelude to IMI, keratin samples were initially taken after teat end sanitization with 70% ethanol, as has been described for the research herd, followed by the collection of secretion samples. A total of 31 unbred and 85 primigravid heifers were sampled in four herds and processed as described, except that species identification of staphylococci was performed using the API® Staph-Trac system (Analytab Products, Plainview, NY).

Intramammary infections were found in 96.9% of heifers and 74.6% of quarters. Heifer mammary glands were infected in an average of 2.8 quarters each. Twenty-nine percent of heifers and 15.1% of quarters showed symptoms of clinical mastitis. Bacteriological status of clinical quarters was as follows: 35% Staph. chromogenes, 22% Staph. aureus, 17% Staph. hyicus, 9% Streptococcus sp., 9% combination of two species of staphylococci or streptococci, 6% no isolation, and 2% Nocardia sp.

The prevalence of IMI in heifers in commercial dairies has been reported previously. Meaney (29) found that prevalence of IMI in heifers at calving ranged between 26 and 42%; 70 to 95% of infected quarters showed clinical symptoms. Oliver (35) sampled heifers in a research herd 14 d pre- and postpartum during a 4-yr period and reported a 64% prevalence of IMI. Pankey et al. (41) diagnosed IMI in 45.5% of heifers (18.7% of quarters) at parturition in 11 herds. Prevalence of IMI in the research herd study (86.1% of quarters) by Boddie et al. (3) was similar to the prevalence of IMI in unbred and primigravid heifers (74.6% of quarters) reported in the study reported by Trinidad et al. (49).

Teat canal colonization occurred in 93.1% of heifers and 70.7% of quarters. A mean of three teat canals was colonized in each heifer. The percentage of quarters with teat canal colonizations was similar to the 70.1% reported in the research herd by Boddie et al. (3) for primigravid heifers. The teat canal and associated tissues are recognized as the primary defense against IMI (31). Bacteria must overcome the teat canal to invade tissues of the mammary gland and to establish infection. Keratin occluding the teat canal lumen is composed largely of lipids and cationic proteins with bactericidal properties (19, 20); however, bacteria are able to survive in keratin (3, 9). Colonization of teat canal keratin may serve as a reservoir for IMI, and colonized bacteria may produce substances that are deleterious to mammary tissue development (9).

Staphylococcus aureus in secretion was isolated from 37.1% of heifers and 14.9% of quarters. Additionally, Staph. aureus was isolated from 22% of quarters with clinical IMI. Teat canal colonizations by Staph. aureus were found in 31% of heifers and 12.3% of quarters. The prevalence of Staph. aureus IMI in unbred and primigravid heifers in Louisiana was higher than expected in these herd surveys (3, 32, 49). However, at the time these surveys were conducted, there were no previously published reports on prevalence of Staph. aureus IMI in this age group of heifers, so a comparison could not be made. Data collected more recently from other sites in the US indicated a lower prevalence of Staph. aureus IMI in unbred and pregnant heifers in Vermont, Washington, and California (14). Apparently, environmental and management factors in Louisiana may promote development of Staph. aureus IMI (3, 32, 49).

Distribution of microorganisms isolated from samples of secretion and teat canal keratin is shown in Table 3. The 3 most common bacterial species isolated from secretions and keratin were Staph. chromogenes, Staph. hyicus, and Staph. aureus. A similar order of
TABLE 3. Distribution of microorganisms isolated from secretion and teat canal keratin samples of 31 unbred and 85 primigravid heifers from four dairy farms.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Secretion (no. quarters)</th>
<th>Secretion (%)</th>
<th>Teat canal (no. quarters)</th>
<th>Teat canal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>55</td>
<td>19.9</td>
<td>54</td>
<td>16.8</td>
</tr>
<tr>
<td>Staphylococcus chromogenes</td>
<td>119</td>
<td>43.1</td>
<td>138</td>
<td>42.9</td>
</tr>
<tr>
<td>Staphylococcus hyicus</td>
<td>67</td>
<td>24.3</td>
<td>81</td>
<td>25.2</td>
</tr>
<tr>
<td>Staphylococcus hominis</td>
<td>2</td>
<td>6.6</td>
<td>4</td>
<td>1.2</td>
</tr>
<tr>
<td>Staphylococcus simulans</td>
<td>2</td>
<td>6.6</td>
<td>4</td>
<td>1.2</td>
</tr>
<tr>
<td>Staphylococcus xylosus</td>
<td>3</td>
<td>1.1</td>
<td>3</td>
<td>.9</td>
</tr>
<tr>
<td>Staphylococcus warneri</td>
<td>1</td>
<td>.3</td>
<td>2</td>
<td>.6</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>1</td>
<td>.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus haemolyticus</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus saprophyticus</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus sp.</td>
<td>1</td>
<td>.3</td>
<td>3</td>
<td>.9</td>
</tr>
<tr>
<td>Streptococcus dysgalactiae</td>
<td>1</td>
<td>.4</td>
<td>2</td>
<td>.6</td>
</tr>
<tr>
<td>Streptococcus sp.</td>
<td>9</td>
<td>3.3</td>
<td>10</td>
<td>3.1</td>
</tr>
<tr>
<td>Nocardia sp.</td>
<td>1</td>
<td>.4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Unidentified</td>
<td>2</td>
<td>3.6</td>
<td>1</td>
<td>.3</td>
</tr>
<tr>
<td>Mixed isolatesf</td>
<td>14</td>
<td>5.1</td>
<td>18</td>
<td>5.7</td>
</tr>
<tr>
<td>Total</td>
<td>276</td>
<td></td>
<td>322</td>
<td></td>
</tr>
</tbody>
</table>

Two species of staphylococci or streptococci were isolated from the same source.


dation from primigravid heifers was reported by Boddie et al. (3). White et al. (55) sampled teat apexes of heifer calves and teat canals from prepuberal heifers and found that Staph. chromogenes was the most frequent bacterial species isolated. Percentage distributions of each microorganism between samples of secretion and teat canal were very similar. As in the study by Boddie et al. (3), teat canal colonization and IMI were correlated. Three different species of staphylococci that were isolated from teat canal keratin samples were not isolated from secretion samples in the study by Trinidad et al. (49). These microorganisms were probably transient colonizations, or they were limited to the teat canal area because of the bactericidal action of keratin or the inability to develop IMI.

SCC and Differential Cell Counts

Mean SCC in secretion from 325 quarter samples was 11.7 x 10^6/ml. Infected (n = 240) and uninfected (n = 85) quarters had secretion SCC of 13.6 x 10^6/ml and 5.7 x 10^6/ml. Distribution of mean SCC associated with the most frequent species and groups of isolates showed that, for the staphylococci, quarters infected with Staph. aureus had the highest SCC (17.3 x 10^6/ml), followed by Staph. chro-
cyte type in secretions of uninfected quarters of dry cows. Sordillo et al. (47) found that macrophage percentages in quarters of dry cows were not affected by IMI, but that neutrophil percentages were significantly higher in infected quarters than in uninfected quarters; however, for heifers, prevalence of leukocyte types was not significantly different between infected and uninfected quarters. The reason for this lack of neutrophil response to natural IMI is unknown. However, intramammary infusion of 5 μg of lipopolysaccharide into unbred heifers elicited a marked neutrophilia into mammary secretions (42); thus, heifers are capable of a neutrophil response. Similarly, the distributions of lymphocytes, macrophages, and neutrophils in mammary secretions from unbred and primigravid heifers were not affected by pregnancy status.

Determination of the origin of IMI and teat canal colonizations in heifers was beyond the scope of these initial studies. However, more stringent mastitis management practices for lactating cows may help to decrease cross-contamination of pathogens between mature cows and heifers. Teat dipping after milking must be continued to reduce pathogens on teat surfaces and possible transfer of organisms from infected cows to heifers by flies. Fly control, a highly effective practice for controlling summer mastitis (12), may help reduce this vector source. Suckling among calves and heifers has been associated with mastitis (45) and should be prevented, particularly if calves are fed mastitic milk. Segregation of pregnant heifers from dry cows may also help to prevent the development of mastitis in heifers. In addition, replacement heifers should be cultured for presence of mastitis-causing bacteria prior to purchase or before entering the milking herd. Therapy for both dry and lactating cows plays a major role in control of mastitis in mature cows; thus, some form of antibiotic treatment could be considered to control IMI in unbred and primigravid heifers. Protection of the developing milk-producing tissues of heifers from bacterial invasion is important to ensure optimal future milk yield.

ANTIBIOTIC TREATMENT OF HEIFERS DURING PREGNANCY

Because of the high incidence of infection, elevated SCC, and prevalence of Staph. aureus, several heifers from each commercial herd surveyed were randomly selected to receive a single intramammary treatment of a penicillin and dihydrostreptomycin product (50). Antimicrobial susceptibility testing demonstrated that 97% of the Staph. aureus isolates were sensitive to 12 antibiotics, including the product selected for treatment (51). Teat ends were sanitized, and a dry cow antibiotic containing 1,000,000 U of penicillin and 1 g of dihydrostreptomycin was infused into all quarters using the partial insertion technique (4). Treatments were made <60 d prior to the calculated calving date. To determine the effect of intramammary treatment on the incidence of mastitis at calving, quarters were sampled prior to treatment to determine bacteriologic and SCC status and then again at calving.

Results showed that 97.1% of treated heifers (73.2% of quarters) were infected at the time of treatment, but, at calving, infected heifers and quarters in the treatment group were reduced to 40 and 34%, respectively (50). Antibiotic residues were limited to two heifers that were treated within 3 wk of calving because estimated dates of parturition were miscalculated, but all quarters were negative after 5 d; 2.9% of treated quarters had antibiotic residues at time of calving. In the untreated control group, 100% of heifers (71.2% of quarters) were infected at initial sampling, and, at calving, mastitis in control heifers was reduced only slightly to 97.4%; infected quarters increased slightly to 77.8%. The mean SCC at calving was also reduced. For treated heifers, SCC decreased significantly (P < .001) from 11,825 x 10^3 ml at treatment to 3439 x 10^3/ml at calving. In the control group, SCC decreased from 11,047 x 10^3/ml to 5594 x 10^3/ml (P > .05).

Staphylococcus aureus was isolated from 11 quarters of 6 treated heifers before antibiotic infusion (45.8%), but, at calving, this organism was isolated from only 1 quarter of 1 heifer (4.2%). For the control group, 18 quarters of 10 heifers were infected with Staph. aureus at treatment (45%). At calving, 6 of the control heifers still had Staph. aureus mastitis in 11 quarters (55%). Thus, the overall incidence of IMI was reduced 60% and that caused by Staph. aureus was reduced over 90%.

From 20 to 26% of quarters with subclinical and clinical Staph. aureus mastitis are typically cured after antibiotic therapy (6, 39).
this study, 90.9% of quarters were cured; thus, antibiotic therapy in heifers was highly effective in eliminating mastitis compared with therapeutic success for lactating cows. Reasons are unclear, but the relatively small udders of heifers may have limited the microorganisms to areas of mammary tissue in which the antibiotic would be present in adequate concentrations to eliminate infection. In addition, scar tissue, common in Staph. aureus infections, may not have formed, which may have permitted the antibiotic to reach all sites of the infecting bacteria.

Antibiotic therapy in heifers is advantageous over treatment of lactating cows because treatment can be performed before calving, daily milk is not discarded, and the risk of antibiotic residues at calving is minimal. In 1 of the commercial herds, heifers that received dry cow therapy during pregnancy produced a mean of 2.5 kg/d more milk over the first 2 mo of lactation than did heifers not receiving therapy. This increase amounted to $42.00 per cow for the first 2 mo of lactation, which would have paid for the cost of four mastitis tubes approximately eightfold.

A more recent study (37) with pregnant heifers using a cephalosporin-based product for nonlactating cows was also successful. Heifers that were either experimentally or naturally infected with Staph. aureus were infused 10 wk prepartum with one dose of 300 mg of a cepapirin benzathine product and were compared with untreated controls infected with Staph. aureus. Results demonstrated that 100% of experimentally induced IMI and 87% of naturally occurring Staph. aureus IMI were eliminated in treated heifers at calving, and cured quarters remained negative at biweekly samples collected 2 mo into lactation. In a continuation of that study (37), quarters remaining infected at calving with Staph. aureus were treated with a lactating cow product containing 200 mg of cepapirin benzathine, but the cure rate was lower (50 to 56%). After antibiotic infusion, SCC in infected quarters that cured spontaneously decreased from $15 \times 10^6$/ml to $4 \times 10^6$/ml 1 wk later and to $700 \times 10^3$/ml at calving. In contrast, none of the untreated control quarters infected with Staph. aureus cured spontaneously by the time heifers calved. Treated heifers in which Staph. aureus IMI were cured yielded a mean of 16.4 kg milk/d, and untreated controls that retained Staph. aureus IMI yielded 14.5 kg/d or 11% less during the first 2 mo of lactation.

Generally, spontaneous cure rates for major mastitis pathogens are low. For example, in a subsequent study on heifer mastitis (38), spontaneous cure rates for Staph. aureus and the environmental streptococci were 9 and 6%, respectively. Thus, treatment is required to cure such infected quarters in these young dairy heifers. New IMI rates in uninfected quarters receiving no therapy over the period from 8 to 10 wk prepartum were very low for most species of bacteria. However, new environmental streptococcal IMI were common in uninfected, untreated heifers. Prophylactic treatment of such quarters prepartum reduced new environmental streptococcal IMI at calving by 93%. Thus, use of nonlactating cow therapy is effective in preventing new IMI as well as curing existing infections.

**OTHER FACTORS TO CONSIDER FOR THE CONTROL OF HEIFER MASTITIS**

In investigations of mastitis in heifers in commercial herds (32), additional parameters were evaluated. For example, overall prevalence of IMI in five herds was approximately twice as high for Jerseys (67.7%) as for Holsteins (35%). Also, heifers that had scabs and abrasions on the teat skin surface, presumably induced by flies, had a higher frequency of IMI (70%) than did those with normal teats (40%). Moreover, herds using some form of fly control (three herds) had a lower percentage of heifers infected with mastitis-causing organisms than those without fly control (two herds), especially for heifers infected with Staph. aureus and Streptococcus sp. (Table 4).

At initial sampling during pregnancy, the frequency of clinical mastitis in infected quarters among heifers in some commercial dairies was 7.5%. At calving, frequency of clinical mastitis increased to 24%, indicating that either the presence of new IMI during the prepartum period led to flare-ups of clinical mastitis at freshening or that chronically infected quarters exhibited clinical flare-ups at this time. In either case, clinically infected quarters in heifers should be controlled prepartum rather than at or after calving.

In one survey, SCC in uninfected quarters of heifers decreased from $7.6 \times 10^6$/ml of
lactating cow product was advantageous be-

Actinomyces pyogenes 0

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Histopathologic changes to developing mam-

mary tissue and markedly elevated SCC sug-

TABLE 4. Prevalence (%) of mastitis in 600 unbred and

primigravid heifers in five herds with (three herds) and

without (two herds) fly control.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Infected heifers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With fly control</td>
</tr>
<tr>
<td>Coagulase-negative</td>
<td>32.9</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>5.6</td>
</tr>
<tr>
<td><em>Streptococcus sp.</em></td>
<td>3.7</td>
</tr>
<tr>
<td>Coliforms</td>
<td>2.2</td>
</tr>
<tr>
<td><em>Actinomyces pyogenes</em></td>
<td>0</td>
</tr>
</tbody>
</table>

lacteal secretion at approximately 2 mo prepartum to 1.5 × 10⁶/ml of colostrum at calving. In infected quarters, SCC decreased from 23.1 × 10⁶/ml prepartum to >4 × 10⁶/ml at calving. This result again indicates the need for infected heifers to be treated or that prophylactic measures be taken so that heifers enter the milking herd with low SCC.

CONCLUSIONS

Prevalence of IMI in unbred and primigravid heifers, especially *Staph. aureus* IMI, was higher than previously realized. Histopathologic changes to developing mammary tissue and markedly elevated SCC suggested that future milk yield may be affected. The treatment of heifers prepartum with a non-lactating cow product was advantageous because the cure rate was higher than during lactation, especially against *Staph. aureus*. There were no milk losses during therapy, the risk of antibiotic residues was minimal, SCC at calving was reduced, and milk yield was increased in successfully treated cows. Treatment is indicated only for herds experiencing a high prevalence of heifers that calve with clinical mastitis caused by *Staph. aureus* or by the environmental streptococci; a treatment program should be developed under the supervision of the herd veterinarian. Residue testing should be carried out before the milk from treated cows is mixed with herd milk.

Thus, use of intramammary infusion products formulated for treating cows at drying off has been successful. Alternatively, systemic administration of antibiotics would be advantageous and would avoid the difficulties associated with restraining the heifers and administering product via the teat canal. Before infusion tubes or systemic injection formulas are designed for treating IMI of dairy heifers, this age group needs to be surveyed nationally to determine whether prevalence of IMI, SCC levels, antimicrobial susceptibility patterns, and influence of season warrant the development of new therapeutic and prophylactic products for mastitis control in heifers.

REFERENCES